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TITLE: Human Leukocyte Antigen (HLA) Genotype as a Contributor to Racial/Ethnic Differences in Breast Cancer: A Population-Based, Molecular Epidemiologic Study

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ABSTRACT

Breast cancer incidence differs across racial/ethnic groups, but known risk factors do not explain all this variation. The human leukocyte antigen (HLA) component of the immune system, coded by highly polymorphic genes whose distribution varies by race/ethnicity, may be a biologically based risk factor for breast cancer and thus may explain some of its racial/ethnic variation. Therefore, for a population-based series of post-menopausal white, black and Hispanic breast cancer cases and controls, we are determining HLA class I (A, B) and class II (DR, DQ) genotypes; whether HLA genotype is related to breast cancer overall; whether associations and prevalence of associated HLA genotypes vary by race/ethnicity, and how much such differences explain racial/ethnic differences in breast cancer incidence; whether HLA associations vary by indicators of prognosis, tumor characteristics, or known breast cancer risk factors. With HLA now typed on all 915 specimens, class I A and B were not strongly associated with breast cancer risk. However, risk increased for whites with A-23 and African-Americans with A-32, and decreased for Hispanics with B-7 after adjustment for age and reproductive risk factors. Continuing analyses will examine associations with other breast cancer risk factors and with HLA class II DR and DQ.

Table of Contents

Introduction:

The incidence and mortality burdens of breast cancer differ markedly across racial/ethnic groups, particularly in post-menopausal women, but known risk factors do not explain all of this variation, or the majority of breast cancers. The human leukocyte antigen (HLA) component of the immune system, encoded by highly polymorphic genes that both vary across racial/ethnic groups and have been related to numerous diseases, has not been much examined for non-viral cancers. Therefore, to examine whether genetically determined aspects of immune function represent biologically-based risk factors for breast cancer and explain a portion of its variation by race/ethnicity, we are taking advantage of already collected DNA and epidemiologic data for a population-based series of post-menopausal white, black and Hispanic breast cancer cases and controls. With the DNA, we used PCR-based, immobilized probe (sequence-specific oligonucleotide) typing to assign HLA genotypes to 915 incident invasive post-menopausal breast cancer cases and post-menopausal controls frequency-matched for age and race/ethnicity. We are now assessing whether HLA genotypes are associated with breast cancer overall and in each of the three racial/ethnic groups by comparing allele or haplotype distributions between cases and controls and quantifying the extent of association with odds ratios. We are also determining if associations differ among the racial/ethnic groups by comparing allele-specific odds ratios and population prevalences of risk-associated alleles, and quantifying the proportion of racial/ethnic incidence differences explained by HLA using the relative attributable risk. As sample sizes permit, we are exploring whether HLA associations relate to tumor characteristics, particularly stage at diagnosis, or known breast cancer risk factors. An association of HLA with racial/ethnic variation in breast cancer occurrence and progression would facilitate a clearer understanding of breast cancer etiology in the group affected and could contribute to more targeted methods of breast cancer prevention. In a clinical setting, HLA type theoretically could be helpful in assessing an individual woman's breast cancer risk profile and, if HLA proved to be linked to breast cancer stage or prognosis, for guiding therapeutic decisions.

Body:

Task 1: Develop study subject database from existing databases

a. *Apply eligibility criteria to the Bay Area Breast Cancer Study (BABCS) study database to select post-menopausal, blood-providing study subjects and extract relevant epidemiologic and specimen-tracking data.*

This task was completed. We identified 426 cases and 489 controls who self-described as post-menopausal in an in-person interview, and for whom DNA was available.

b. *Link study patients to Greater Bay Area Cancer Registry database to obtain demographic, clinical and tumor characteristics.*

This task was completed, yielding a study database.

c. *Install linked study subject database into study tracking database, creating study identification (ID) numbers.*

This task was completed once IRB approval was received from the funding agency permitting us to work with identifiable human materials.

Task 2: Obtain DNA samples from storage at USC

a. *Transmit electronic file of study-subject BABCS tracking ID numbers to Dr. Engles' lab at USC.*

This task was completed.

b. *Request DNA for each patient be transmitted in 96-well trays to Dr. Erlich's lab, labeled only by unique specimen ID number.*

This task was completed. DNA samples, each comprising 1 microgram of DNA that had been dried down, were transmitted from Dr. Ingles' lab to Dr. Erlich's staff in 11 plates.

c. *Track DNA specimen transmission.*

This task was completed. Transmission of the DNA was tracked and its receipt was acknowledged by Dr. Erlich's staff.

Task 3: HLA-type DNA specimens

a. *Amplify class I (A,B) and class II (DQ,DR) loci.*

This task was completed.

b. *Type using immobilize probe linear arrays.*

This task was completed.

c. *Scan probe reactivity patterns and convert patterns to genotype.*

This task was completed.

d. *Record assay results on Excel spreadsheet and transmit back to NCCC.*

This task was completed, and a spreadsheet with all genotyping results was transmitted to NCCC. At present, Class I results are being organized by HLA haplotype by Dr. Erlich and his staff, and will be transmitted when this work is completed.

Task 4: Create study database (months 19)

a. *Merge study database, including interview and registry data, with HLA typing data*

This task was completed.

b. *Link study database to Greater Bay Area Cancer Registry database to obtain most current patient vital status.*

This task has not yet been completed. Our first analytic priority was to look at associations of HLA with race/ethnicity, with consideration to the impact of known breast cancer risk factors. Once this work is complete, we will evaluate the impact of HLA type on outcome after breast cancer; at that time, we will obtain the most current patient vital status.

c. *Strip database of all subject identifiers.*

This task was completed for the analytic database we are presently using. Dr. John retains the key so that we can return to her for additional variables and so that she can facilitate linkage to the cancer registry database for vital status.

Task 5: Statistical analysis (months 20-23)

a. *Compare allele frequencies for HLA-A, -B, -DQ and -DR separately for each race.*

This task was completed for Class I (A and B).

b. *Compute odds ratios and 95% confidence intervals*

This task was completed for Class I (A and B).

c. *Compare across racial/ethnic groups HLA associations significant in any racial/ethnic group.*

This task was completed for Class I (A and B)

d. Examine whether relationships are confounded by other epidemiologic or tumor features.

This task has been partially completed for Class I (A and B); we have examined three reproductive factors but have not yet looked at the effect of hormone use or family history, or looked at tumor features.

e. Conduct logistic regression to predict breast cancer risk associated with the alleles of interest with control for confounders

This task was completed for Class I (A and B) for the reproductive variables mentioned above.

Task 6: Summarize study findings for presentation and submission for publication in literature (months 23-24)

Study findings have been summarized for the Era of Hope abstract, poster and platform presentation in Philadelphia, June 8-11. Associated PowerPoint files are attached.

Key Research Accomplishments:

1) Develop study subject database from existing databases
2) Link study patients to Greater Bay Area Cancer Registry database to obtain demographic, clinical and tumor characteristics.
3) Install linked study subject database into study tracking database, creating study identification (ID) numbers.
4) Transmit electronic file of study-subject tracking ID numbers to Dr. Engles' lab at USC, where the DNA resides.
5) Request DNA for each patient be transmitted in 96-well trays to Dr. Erlich's lab, labeled only by unique specimen ID number.
6) Transmit DNA in 11 96-well plates.
7) Track DNA specimen transmission.
8) Amplify class I (A and B) and class II (DR and DQ) loci for 915 samples.
9) Type using immobilize probe linear arrays.
10) Scan probe reactivity patterns and convert patterns to genotype.
11) Record assay results on Excel spreadsheet and transmit back to NCCC.
12) Create study database by merge HLA database with interview and registry data and stripping database of all subject identifiers so analyses could begin.
13) Undertaking and completing most of the statistical analysis for Class I alleles, by comparing allele frequencies for HLA-A, and -B separately for each race; computing odds ratios and 95% confidence intervals adjusted for age; comparing across racial/ethnic groups those HLA associations significant in any racial/ethnic group; examining whether relationships are confounded by reproductive factors (age at menarche, at a first full-term pregnancy, duration of lactation); and conducting logistic regression including age and these variables.

Reportable Outcomes:

1) Abstract (Bugawan TL et al.) submitted to the American Society of Histocompatibility and Immunogenetics for the October, 2005 meeting
2) Abstract (Glaser SL et al.) as required for Era of Hope meeting, June 8-11, 2005, Philadelphia
3) Poster as required for Era of Hope meeting, June 8-11, 2005, Philadelphia
4) Invited platform presentation, Era of Hope meeting, June 9, 2005, Philadelphia

Conclusions:

Class I A:

As presented in the attached PowerPoint files, for post-menopausal breast cancer overall there was a suggestive association with HLA class I A only for A-23, although it was not significant after correction for multiple comparisons; for the adjusted odds ratio (OR) (OR=1.6, 95% confidence interval (CI) 1.0 – 2.5), the

lower confidence limit included 1. For breast cancer by race/ethnicity, Class I A allele associations differed. There were suggestive associations for A-23 in whites and for A-32 in African-Americans, although these were not statistically significant after adjustment for multiple comparisons. In phenotypic analyses looking at breast cancer risk, odds ratios adjusted for age and reproductive factors were significant for A-23 in whites (OR=4.1, 95% CI 1.1 – 15.2), A-32 in African-Americans (OR=10.0, 95% CI 1.2 – 82.2), and A-01 in Hispanics (OR=0.5, 95% CI 0.3 – 0.99). The risks were strong, but the confidence intervals were wide.

Class I B:

For Class I B alleles and breast cancer overall, there were suggestive allele-specific associations for B-13, B-39 and B-50, but they were not significant after correction for multiple comparisons. However, linear regression revealed a significant reduced risk of breast cancer in women positive for B-39 (adjusted OR=0.5, 95% CI 0.3 – 0.9). Across racial/ethnic groups, associations between Class I B alleles and breast cancer also differed. There were suggestive allele-specific associations for B-44 in African-Americans and B-7 in Hispanics, but they were not statistically significant after adjustment for multiple comparisons. After adjustment for age and reproductive factors, linear regression showed a significant, reduced breast cancer risk for B-07 in Hispanic women (OR=0.5, 95% CI 0.2 – 0.9).

Thus far in our analysis, we tentatively conclude that HLA Class I A and B alleles may not be strongly related to breast cancer risk in post-menopausal women. However, there are suggestions of associations and, moreover, apparent differences in associations by race/ethnicity. Our data indicate that breast cancer risk may be increased for white women with A-23 and African-Americans with A-32 and reduced for Hispanic women with B-7. These risk patterns persist after adjustment for age and reproductive risk factors. Therefore, they do support a possible role of HLA or linked loci in contributing to some part of racial/ethnic variation in breast cancer incidence, and thus HLA may be involved in immunosurveillance and/or hormonal pathways related to breast cancer.

We are presently continuing to look at how associations are affected by other breast cancer risk factors and by tumor characteristics. We are planning to look at allelic variants for the associated alleles and conduct haplotype analyses looking at A-B haplotypes. We also are going to repeat all these analyses for class II DR and DQ, and for DR-DQ haplotypes.

References:

None yet.

Appendices:

Abstract submitted to the American Society of Histocompatibility and Immunogenetics for the October, 2005 meeting
Poster and study results slides for platform presentation at Era of Hope meeting in Philadelphia, June 9, 2005 Abstract/ASHI-05

Human Leukocyte Antigen Genotype and Racial/Ethnic Differences in Breast Cancer: Preliminary Results for Class I

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BACKGROUND

Among post-menopausal women, breast cancer incidence differs markedly across racial/ ethnic groups, but known risk factors do not explain all of this variation. A novel factor that might be related is the immune system, particularly the human leukocyte antigen (HLA) component, because it is encoded by highly polymorphic genes that both vary across racial/ethnic groups and have been associated with diseases, including some cancers. Previous studies of breast cancer and serologically determined HLA type were inconsistent, but a few recent, if limited, studies found strong HLA associations, suggesting that genetically determined aspects of immune function may represent biologically based risk factors for breast cancer and thus could explain a portion of its variation by race/ethnicity. Yet, this association has never been examined in a well-designed study that is population-based, addresses racial/ethnic variation in breast cancer incidence, considers confounding by known risk factors, and utilizes DNA-based methods for assigning HLA type.

RESEARCH OBJECTIVES

To determine: 1) if HLA class I (A and B) and class II (DR and DQ) genotypes are associated with invasive breast cancer in white, African-American, and Hispanic post-menopausal women; 2) if HLA-breast cancer associations differ among these racial/ethnic groups.

METHODS

Subjects: This study used DNA and epidemiologic data from post-menopausal participants in a previously conducted population-based case-control study. For that study, ***cases*** were women identified by the Greater Bay Area Cancer Registry as having incident invasive breast cancer diagnosed in 5/97-4/99 at ages 35-79. Of 4,603 living cases, 3,799 (83%) were screened for self-reported race/ethnicity and breast cancer history. Of 1,041 without prior breast cancer, all African-Americans and Hispanics and a 10% sample of whites were eligible for an in-person interview, completed by 929 (89%). A year later, biospecimens were obtained from 277 (90%) whites, 250 (85%) African-Americans, and 287 (88%) Hispanics. ***Controls*** were identified by random-digit dialing and frequency-matched to cases on race/ethnicity and age. 1,274 of 1,470 (87%) completed screening for breast cancer and race/ethnicity. Of the 1,208 eligibles, 1,046 (87%) were interviewed. Biospecimens were obtained for 299 (93%) whites, 255 (84%) African-Americans, and 357 (85%) Hispanics.

HLA genotyping: Class I (A, B) and II (DRB1, DQB1) were typed based on PCR amplification with biotinylated primers, hybridization to an immobilized (SSO) probe linear array, and detection of probe reactivity pattern with streptavidin-HRP. The developed strips were scanned using a flat bed document scanner and software to interpret the probe binding pattern and to assign the sample HLA genotype.

Statistical analysis: Allele-specific analyses were conducted using cross-tabulations with X^2 and Fisher exact tests. To estimate breast cancer risk associated with suggestive alleles (carriers = hetero- and homozygotes), logistic regression was used to produce odds ratios (OR) and 95% confidence intervals (CI) adjusted for diagnosis age, age at menarche (<12 yrs., ≥12 yrs.), lactation (nulliparous, <5 mos., ≥5 mos.), and age at 1st full-term pregnancy (nulliparous or ≥30 yrs., other); race was included in the initial but not final all-race models.

PRELIMINARY RESULTS

Table 1. N of post-menopausal subjects by race/ethnicity and case-control status

Subjects	Whites	African-Americans	Hispanics
Cases	152	134	140
Controls	162	140	187

DNA typing was successful for ~99% of subjects.

HLA Class I A and breast cancer

Overall: Suggestive allele-specific association for **A-23** (p=0.05);

By race/ethnicity: Suggestive allele-specific associations (**Table 2**) and adjusted OR's (**Table 3**) for **A-23** in whites, **A-32** in African-Americans, **A-01** in Hispanics.

HLA Class I B and breast cancer

Overall: Suggestive allele-specific associations for **B-13** (p=0.03), **B-39** (p=0.02), and **B-50** (p=0.04); significant adjusted OR for **B-39** (**Table 4**).

By race/ethnicity: Suggestive allele-specific associations for **B-44** in African-Americans and **B-7** in Hispanics (**Table 5**). Adjusted OR's: 1.8, 95% CI 0.9 – 3.3; **0.5, 95% CI 0.2 – 0.9**, respectively.

Multiple comparisons: No Class I allele-specific associations were statistically significant after Bonferroni-type correction for multiple comparisons.

Table 2. Relative frequency distributions of Class I A alleles by race/ethnicity

A Allele	Whites			African-Americans			Hispanics		
	Cases N*=304	Controls N*=318	p†	Cases N*=266	Controls N*=280	p†	Cases N*=276	Controls N*=368	p†
1	15.5	13.2	0.42	5.3	5.7	0.82	6.2	10.1	0.08
2	27.3	27.7	0.92	20.7	21.4	0.83	30.8	28.8	0.58
3	15.5	13.2	0.42	6.4	6.1	0.90	8.7	7.9	0.71
11	5.6	8.2	0.20	1.5	1.4	1.00	3.3	4.9	0.31
23	**4.0**	**0.9**	**0.01**	9.0	8.2	0.74	2.9	1.6	0.27
24	5.6	9.4	0.07	2.6	3.6	0.53	13.4	13.3	0.97
25	2.0	1.9	0.94	-	0.7	0.50	0.4	0.8	0.64
26	2.6	3.1	0.70	1.9	3.2	0.32	2.9	2.5	0.72
29	3.6	4.1	0.76	5.3	3.2	0.23	5.1	3.0	0.18
30	3.6	2.8	0.58	10.2	13.9	0.18	5.4	3.5	0.24
31	4.0	2.2	0.21	0.8	1.1	1.00	5.8	5.2	0.73
32	3.3	4.7	0.36	**3.8**	**0.4**	**0.005**	1.1	3.0	0.10
33	2.0	1.3	0.54	6.8	4.3	0.20	2.9	2.2	0.56
34	0.0	0.3	1.00	6.0	4.6	0.50	-	-	
36	-	-		1.9	1.8	1.00	-	-	
66	-	0.6	0.17	3.4	3.2	0.91	0.7	0.5	1.00
68	4.9	4.7	0.90	10.5	8.9	0.53	9.8	12.2	0.33
69	-	-		-	-		-	0.3	1.00
74	0.7	1.6	0.45	3.8	7.5	1.00	0.7	0.3	0.58
80	-	-		0.4	0.7	0.59	-	-	

* N = number of alleles † p not corrected for multiple comparisons

Table 3. Adjusted* odds ratios for suggestively associated Class I A alleles, by race/ethnicity *for age and reproductive risk factors

Allele	Whites		African-Americans		Hispanics	
	Odds ratio	95% CI	Odds ratio	95% CI	Odds ratio	95% CI
A-01	1.2	0.7 – 2.0	1.0	0.5 – 2.2	**0.5**	**0.3 – 0.99**
A-23	**4.1**	**1.1 - 15.2**	1.1	0.6 – 2.1	1.6	0.5 – 5.0
A-32	0.7	0.3 – 1.6	**10.0**	**1.2 - 82.2**	0.3	0.1 – 1.1

Table 4. Adjusted* odds ratios for suggestively associated Class I B alleles, all races/ethnicities combined *for age and reproductive risk factors

Allele	Odds ratio	95% CI
B-13	0.5	0.2 – 1.0
B-39	**0.5**	**0.3 – 0.9**
B-50	0.4	0.2 – 1.0

Table 5. Relative frequency distributions of Class I B alleles by race/ethnicity

B Allele	Whites			African-Americans			Hispanics		
	Cases N*=304	Controls N*=320	p†	Cases N*=266	Controls N*=280	p†	Cases N*=278	Controls N*=368	p†
7	14.8	10.9	0.15	10.2	6.4	0.11	**4.3**	**8.2**	**0.05**
8	10.9	10.6	0.93	3.4	2.9	0.72	4.0	4.1	0.94
13	1.6	3.8	0.11	0.8	1.8	0.45	1.1	2.2	0.37
14	4.0	3.8	0.90	3.4	2.5	0.54	3.2	4.4	0.47
15	7.6	5.6	0.33	13.2	12.1	0.72	8.3	5.2	0.11
18	2.6	2.8	0.89	4.9	4.6	0.89	4.7	3.3	0.36
27	3.3	4.4	0.48	1.1	2.1	0.51	2.5	3.8	0.36
35	7.9	10.6	0.24	5.3	7.1	0.36	17.6	13.3	0.13
37	-	0.9	0.25	0.8	0.7	1.00	1.1	1.4	1.00
38	1.3	1.6	1.00	0.4	1.1	0.34	1.8	2.5	0.58
39	1.0	3.1	0.06	1.5	1.1	0.72	5.4	9.0	0.09
40	6.6	6.6	0.99	1.9	2.1	0.83	13.7	9.2	0.08
41	1.0	0.3	0.36	0.8	1.1	1.00	1.4	0.5	0.41
42	-	-		4.1	7.1	0.13	1.1	0.8	1.00
44	16.8	15.6	1.00	**12.4**	**7.1**	**0.04**	6.5	6.5	0.98
45	0.7	0.6	0.96	4.1	5.0	0.63	0.4	2.2	0.09
47	-	0.3	1.00	0.4	-	0.49	0.7	1.1	0.70
48	0.3	-	0.49	-	-		2.5	2.5	0.95
49	2.6	1.3	0.21	2.3	2.1	0.93	2.5	3.0	0.72
50	0.7	1.9	0.29	0.4	1.8	0.22	1.4	2.2	0.49
51	7.2	5.3	0.32	3.4	2.1	0.38	5.0	5.4	0.82
52	1.0	1.6	0.73	0.8	1.8	0.45	4.0	1.9	0.12
53	1.3	1.6	1.00	9.8	11.8	0.45	2.2	3.3	0.40
54	0.3	-	0.49	-	-		-	-	
55	2.6	2.2	0.72	0.8	0.7	1.00	0.4	-	0.43
56	0.3	0.9	0.62	-	-		-	0.8	0.26
57	2.6	3.1	0.71	3.0	5.0	0.24	1.1	2.2	0.29
58	1.0	0.6	0.68	8.3	6.4	0.41	1.8	0.8	0.30
73	-	-		-	-		0.4	-	0.43
78	-	-		1.5	1.1	0.72	0.7	0.3	0.58
81	-	-		1.1	1.4	1.00	0.4	-	0.43
82	-	-		0.4	0.7	1.00	-	0.3	1.00

* N = number of alleles † p not corrected for multiple comparisons

CONCLUSIONS

Preliminary conclusions: In this exploratory study, DNA-typed HLA class I alleles were not strongly associated with breast cancer risk in post-menopausal women. Associations did differ by race/ ethnicity: breast cancer risk appeared increased for whites with A-23 and African-Americans with A-32, and decreased for Hispanics with B-7 after adjustment for age and reproductive risk factors, supporting a possible role of HLA or linked loci in explaining racial/ ethnic variation in breast cancer incidence, and in immunosurveillance and/or hormonal pathways related to breast cancer. Possible study limitations include potential survival bias, as well as statistical power too low to: 1) detect associations, given multiple comparisons and low prevalence of many alleles; 2) examine interactions with breast cancer risk factors; and 3) compare risks among HLA allele heterozygotes and homozygotes. Continuing analyses will examine associations with other breast cancer risk factors and with HLA class II DR and DQ.

Supported in part by DAMD17-03-0430 (Dr. Glaser) and NCI R01 CA77305 (Dr. John)

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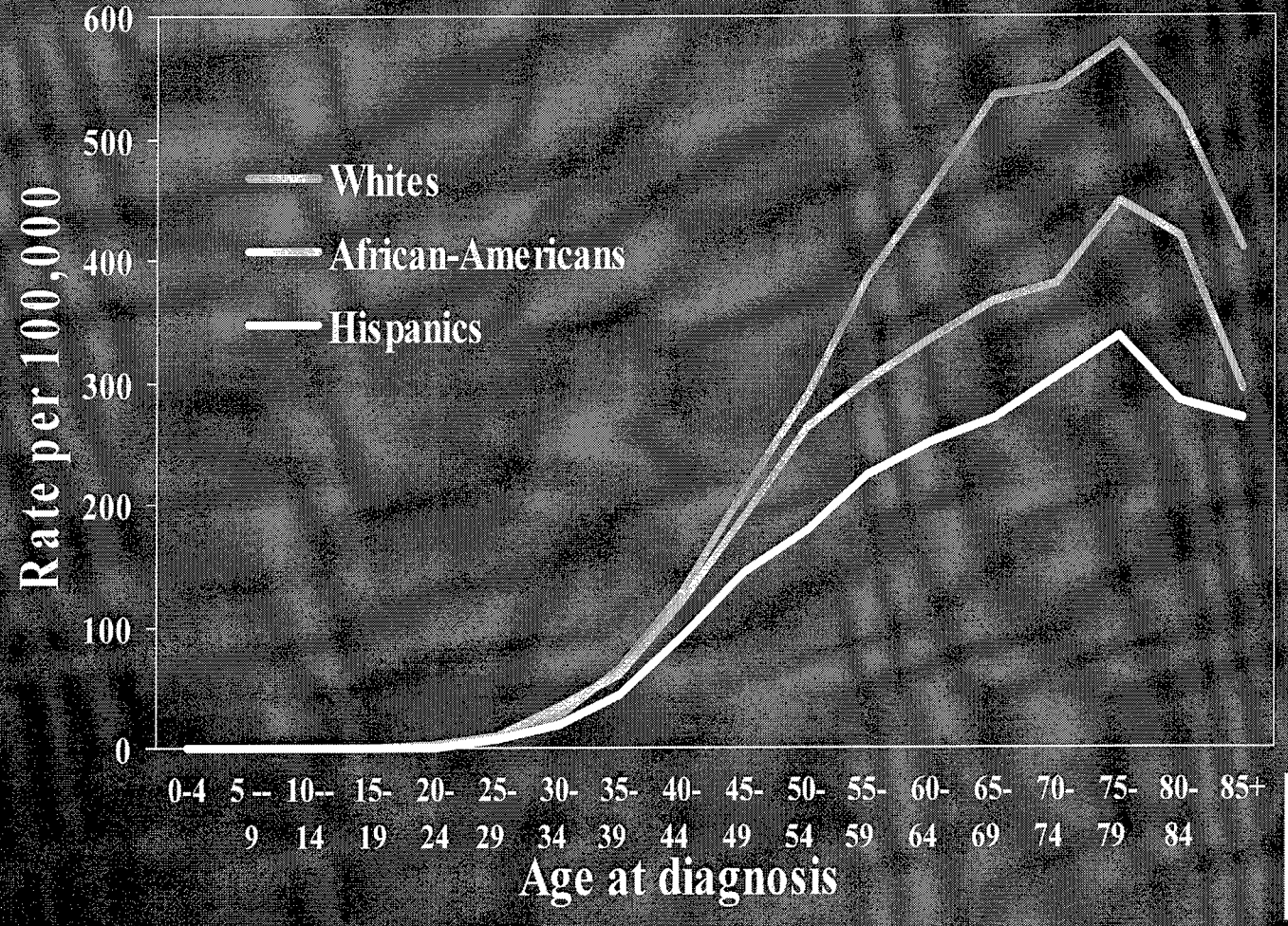
Invasive Breast Cancer Incidence Rates, California, 1998-2002



Invasive Breast Cancer Incidence Stage, California, 1998-2002

AJCC Stage	Whites	African-Americans	Hispanics
Stage I	50%	39%	43%
Stage IV	4%	6%	5%

Breast cancer and race/ethnicity

- differences may be based in biology
- source of hypotheses about novel biological risk factors
- immune system may play a role in risk
- human leukocyte antigen (HLA)
  - part of immune system
  - presenting antigens to T-cells
  - surveillance of tumors
  - class I (A, B), class II (DR, DP, DQ)

HLA

- highly polymorphic across individuals
- associated with various diseases
  - cancers (viral, non-viral (lung, brain)
- highly polymorphic among racial/ethnic groups
  - in allele frequencies
  - unique occurrence of some alleles in specific groups

Breast cancer and HLA

- numerous early studies of breast cancer associated with HLA type
  - examined HLA class I and II
  - serologic typing (not as precise as genotyping)
  - significant but inconsistent findings
- Chaudhuri et al., 2000
  - strong significant associations of breast cancer risk with class II based on genotyping
  - white breast cancer patients < age 40 at diagnosis and white controls

Research question and strategy

- examine association of HLA class I and II genotype with invasive breast cancer in post-menopausal women
  - overall
  - in racial/ethnic variation
- DNA and epidemiologic data from a population-based, San Francisco Bay Area, case-control study of breast cancer conducted by Dr. Esther John, NCCC

Study subjects: breast cancer cases

- invasive breast cancers (n=4033)
- identified from Greater Bay Area Cancer Registry
  - White, African-American, or Hispanic
  - aged 35-79
  - first diagnosed between mid 1997 and mid 1999
- 83% screened by telephone regarding self-reported race/ethnicity and prior breast cancer
- 89% of all African-American, Hispanics, 10% sample of whites interviewed in person
- one year later, biospecimens obtained from:
  - 90% whites
  - 85% African-Americans
  - 88% Hispanics

Study subjects: controls

- identified by random-digit dialing
- frequency-matched to cases on race/ethnicity and 5-year age group
- 87% of 1,470 screened by telephone regarding self-reported race/ethnicity and prior breast cancer
- 87% completed in-person interview
- biospecimens provided by:
  - 93% whites
  - 84% African-Americans
  - 85% Hispanics

Study subjects by race/ethnicity and case-control status

Subjects	Whites	African-Americans	Hispanics
Cases	152	134	140
Controls	162	140	187

HLA genotyping

- in DNA specimens
- PCR-SSO immobilized linear array probe technology
- successful for >99% of subjects

Results
Class I A allele-specific
All women

- for breast cancer overall, suggestive association only for:
  - HLA A-23 (p=0.05)
- not significant after Bonferroni correction for multiple comparisons (requiring $p \leq 0.0025$)
- adjusted OR=1.6, 95% CI 1.0 – 2.5

Distribution, HLA-A alleles by race/ethnicity (1)

A Allele	Whites			African-American			Hispanics		
	Cases N=304	Controls N=318	p	Cases N=266	Controls N=280	p	Cases N=276	Controls N=368	p
1	15.5	13.2	0.42	5.3	5.7	0.82	6.2	10.1	0.08
2	27.3	27.7	0.92	20.7	21.4	0.83	30.8	28.8	0.58
3	15.5	13.2	0.42	6.4	6.1	0.90	8.7	7.9	0.71
11	5.6	8.2	0.20	1.5	1.4	1.00	3.3	4.9	0.31
23	**4.0**	**0.9**	**0.01**	9.0	8.2	0.74	2.9	1.6	0.27
24	5.6	9.4	0.07	2.6	3.6	0.53	13.4	13.3	0.97
25	2.0	1.9	0.94	-	0.7	0.50	0.4	0.8	0.64
26	2.6	3.1	0.70	1.9	3.2	0.32	2.9	2.5	0.72
29	3.6	4.1	0.76	5.3	3.2	0.23	5.1	3.0	0.18
30	3.6	2.8	0.58	10.2	13.9	0.18	5.4	3.5	0.24

Distribution, HLA-A alleles by race/ethnicity (2)

A Allele	Whites			African-American			Hispanics		
	Cases N=304	Controls N=318	p	Cases N=266	Controls N=280	p	Cases N=276	Controls N=368	p
31	4.0	2.2	0.21	0.8	1.1	1.00	5.8	5.2	0.73
32	3.3	4.7	0.36	**3.8**	**0.4**	**0.005**	1.1	3.0	0.10
33	2.0	1.3	0.54	6.8	4.3	0.20	2.9	2.2	0.56
34	0.0	0.3	1.00	6.0	4.6	0.50	-	-	
36	-	-		1.9	1.8	1.00	-	-	
66	-	0.6	0.17	3.4	3.2	0.91	0.7	0.5	1.00
68	4.9	4.7	0.90	10.5	8.9	0.53	9.8	12.2	0.33
69	-	-		-	-		-	0.3	1.00
74	0.7	1.6	0.45	3.8	7.5	1.00	0.7	0.3	0.58
80	-	-		0.4	0.7	0.59	-	-	

Results
Class I A
Adjusted* Odds Ratios

	Whites		African-Americans		Hispanics	
A Allele	Odds ratio	95% CI	Odds ratio	95% CI	Odds ratio	95% CI
A-01	1.2	0.7 – 2.0	1.0	0.5 – 2.2	**0.5**	**0.3 – 0.99**
A-23	**4.1**	**1.1 - 15.2**	1.1	0.6 – 2.1	1.6	0.5 – 5.0
A-32	0.7	0.3 – 1.6	**10.0**	**1.2 - 82.2**	0.3	0.1 – 1.1

*adjusted for age at diagnosis, age at menarche, lactation, age 1st full-term pregnancy

Results
Class I B allele-specific
All women

- for breast cancer overall, suggestive allele-specific associations for:
  - B-13 (1.2% vs 2.6%, p=0.03)
  - B-39 (2.6% vs 4.8%, p=0.02)
  - B-50 (0.8% vs 2.0%, p=0.04)
- not significant after Bonferroni correction for multiple comparisons ($p \leq 0.0016$)
- significant reduced risk for B-39:
  - adjusted OR=0.5, 95% CI 0.3 – 0.9

Distribution, HLA-B alleles (1)

B Allele	Whites			African-American			Hispanics		
	Cases N=304	Controls N=320	p	Cases N=266	Controls N=280	p	Cases N=278	Controls N=368	p
7	14.8	10.9	0.15	10.2	6.4	0.11	**4.3**	**8.2**	**0.05**
8	10.9	10.6	0.93	3.4	2.9	0.72	4.0	4.1	0.94
13	1.6	3.8	0.11	0.8	1.8	0.45	1.1	2.2	0.37
14	4.0	3.8	0.90	3.4	2.5	0.54	3.2	4.4	0.47
15	7.6	5.6	0.33	13.2	12.1	0.72	8.3	5.2	0.11
18	2.6	2.8	0.89	4.9	4.6	0.89	4.7	3.3	0.36
27	3.3	4.4	0.48	1.1	2.1	0.51	2.5	3.8	0.36
35	7.9	10.6	0.24	5.3	7.1	0.36	17.6	13.3	0.13
37	-	0.9	0.25	0.8	0.7	1.00	1.1	1.4	1.00
38	1.3	1.6	1.00	0.4	1.1	0.34	1.8	2.5	0.58
39	1.0	3.1	0.06	1.5	1.1	0.72	5.4	9.0	0.09
40	6.6	6.6	0.99	1.9	2.1	0.83	13.7	9.2	0.08
41	1.0	0.3	0.36	0.8	1.1	1.00	1.4	0.5	0.41
42	-	-		4.1	7.1	0.13	1.1	0.8	1.00
44	16.8	15.6	1.00	**12.4**	**7.1**	**0.04**	6.5	6.5	0.98

Distribution, HLA-B alleles (2)

B Allele	Whites			African-American			Hispanics		
	Cases	Controls	p	Cases	Controls	p	Cases	Controls	p
45	0.7	0.6	0.96	4.1	5.0	0.63	0.4	2.2	0.09
47	-	0.3	1.00	0.4	-	0.49	0.7	1.1	0.70
48	0.3	-	0.49	-	-		2.5	2.5	0.95
49	2.6	1.3	0.21	2.3	2.1	0.93	2.5	3.0	0.72
50	0.7	1.9	0.29	0.4	1.8	0.22	1.4	2.2	0.49
51	7.2	5.3	0.32	3.4	2.1	0.38	5.0	5.4	0.82
52	1.0	1.6	0.73	0.8	1.8	0.45	4.0	1.9	0.12
53	1.3	1.6	1.00	9.8	11.8	0.45	2.2	3.3	0.40
54	0.3	-	0.49	-	-		-	-	
55	2.6	2.2	0.72	0.8	0.7	1.00	0.4	-	0.43
56	0.3	0.9	0.62	-	-		-	0.8	0.26
57	2.6	3.1	0.71	3.0	5.0	0.24	1.1	2.2	0.29
58	1.0	0.6	0.68	8.3	6.4	0.41	1.8	0.8	0.30
73	-	-		-	-		0.4	-	0.43
78	-	-		1.5	1.1	0.72	0.7	0.3	0.58
81	-	-		1.1	1.4	1.00	0.4	-	0.43
82	-	-		0.4	0.7	1.00	-	0.3	1.00

Results
Class I B
Adjusted Odds Ratios

- adjusted odds ratios significant for:
  - B-07 in Hispanics
  - OR=0.5, 95% CI 0.2 – 0.9

Breast cancer and HLA
Preliminary Conclusions

- class I alleles not strongly related to breast cancer risk in post-menopausal women
- suggestion of associations
- associations differ by race/ ethnicity, with risk:
  - ↑ for whites with A-23
  - ↑ for African-Americans with A-32
  - ↓ for Hispanics with B-7
  - after adjustment for age and reproductive risk factors
- possible role of HLA or linked loci
  - contributing to racial/ethnic variation in breast cancer incidence
  - in immunosurveillance
  - hormonal pathways related to breast cancer

Breast cancer and HLA
Study strengths

- population-based
- ethnically matched controls
- good response rates for data collection
- availability of DNA and epidemiologic data
- state-of-the-art HLA genotyping

Breast cancer and HLA
Study limitations

- statistical power too low to
  - detect associations
    - multiple comparisons
    - low prevalence of many alleles
  - examine interactions with breast cancer risk factors
  - compare risks among HLA allele heterozygotes and homozygotes
- possible survival bias

Breast cancer and HLA
Next steps

- associations with:
  - other breast cancer risk factors (HRT)
  - tumor characteristics (stage, grade, ER status)
- allelic variants for associated alleles
- haplotype analyses (A-B)
- all analyses for class II DR and DQ

Abstract/ASHI-05

Unusual DRB1-DQA1-DQB1 haplotypes in Caucasians, Hispanics and African Americans using DRB1 high resolution, DQA1/DQB1 co-amplification and HLA-A and B PCR/SSO linear arrays. TL Bugawan, J Ching, SL Glaser**, EM John**,CA Clarke**,T Harasty**, M Agleham*, and H Erlich, Department of Human Genetics, Roche Molecular Systems, Alameda, CA, *Children's Hospital Research Institute, Oakland, CA, and **Northern California Cancer Center, Fremont, CA.

A specific PCR amplification of the DRB1 locus was accomplished using eight 5' and one 3' primers. Hybridization of the biotinylated PCR products to a panel of 81 SSOP in a linear array format, which allows high resolution typing without separate amplification of DRB1 alleles, was used to genotype 155 Caucasians, 182 Hispanics and 140 African Americans. These samples were also typed for the DQA1, DQB1, A and B loci. The DQ typing was done using a co-amplification of DQA1/DQB1 with locus specific primers. Bw4 and Bw6 group specific primers were used for high resolution typing of HLA-B. Greater DR-DQ haplotype diversity was observed among African Americans and Hispanics than Caucasian population groups: 26 haplotypes (n>4), in Hispanics, 33 in African Americans and 18 in Caucasian were observed This increased DR-DQ haplotype diversity may be attributable to admixture as well as to the greater genetic diversity generally observed among populations of African descent. Analysis of DRB1 -DQB1 haplotypes revealed some rare "unusual" haplotypes among African American and Hispanic populations not observed among Caucasians. The DRB1*1303-DQA1*0201-DQB1*0201, is present in both Hispanics, and African American. DRB1*1302-DQA1*0301-DQB1*0201 haplotype is present in African American but not in Hispanics while DRB1*1402-DQA1*0201-DQB1*0201 is seen only in Hispanics. Another unusual haplotype seen only in African Americans in this study is DRB1*1503-DQA1*0301-DQB1*0201. These unusual haplotypes were presumably generated by recombination between the DQA1 and the DRB1 loci.